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(71) Applicant: CARY PHARMACEUTICALS INC. [US/US]; Suite 700, 7200 Wisconsin Avenue, Bethesda, MD 20814 (US).

(72) Inventor: WILCOX, Christopher, S.; Suite 700, 7200 Wisconsin Avenue, Bethesda, MD 20814 (US).

(74) Agent: HULINA, Amy, L.; Morgan, Lewis & Bockius LLP, 1800 M Street, NW, Washington, DC 20036 (US).

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(54) Title: HIGH DOSE FOLIC ACID FOR THE TREATMENT OF HYPERHOMOCYSTEINEMIA

(57) Abstract: The present invention encompasses methods of treating patients with hyperhomocysteinemia, caused for example by end stage renal disease. The invention also includes related pharmaceutical compositions containing a folate, vitamins and other homocysteine modulating agents which treat severe hyperhomocysteinemia. Specific combinations and dosage levels of folic acid and other vitamins are disclosed. These compositions are also contemplated to lessen the incidence and reduce the complications of cardiovascular and vascular diseases, and blood coagulation problems associated with this group of patients.

High Dose Folic Acid for the Treatment of Hyperhomocysteinemia

FIELD OF THE INVENTION

This invention relates to compositions and methods for treating hyperhomocysteinemia in patients with compromised renal function, especially end stage renal disease (ESRD) patients receiving hemodialysis.

BACKGROUND OF THE INVENTION

Patients with end stage renal disease (ESRD) receiving hemodialysis therapy have an alarming mortality rate of approximately 22% per annum (Fenton *et al.*, 1997 Am. J. Kidney Dis. 30: 334), with the most frequent causes of death being stroke and myocardial infarction. Patients with chronic renal insufficiency and especially those with end stage renal disease (ESRD) have a significantly increased incidence of cardiovascular disease when compared to the general population. (Ritz *et al.*, 1993 Am. J. Kidney Dis. 21 (suppl.2):113-118,Bostom *et al.*, 1997 Arterioscler. Thromb. Vasc. Biol. 17: 2554). Risk factors for cardiovascular disease in the general population include hypertension, dyslipidemia, obesity and smoking. These risk factors have not, however, proven to be reliable predictors of cardiovascular disease in patients with chronic renal insufficiency. Non-traditional cardiovascular risk factors currently considered responsible for ESRD mortality include lipoprotein (a), oxidative stress, elevated plasma levels of dimethylarginine and homocysteine (Gris *et al.*, 1994 Kidney Int. 46: 807; Cressman *et al.*, 1992 Circ. 86: 475; and Paul *et al.*, 1993 Nephron. 64: 106) (2-7).

More than 90% of patients on dialysis have elevated plasma concentrations of homocysteine (Hultberg *et al.*, 1993 Clinical Nephrology 40(4):230-234). Homocysteine (t-

Hcy) is a non-protein amino acid that is the transmethylation product of methionine. Homocysteine may be re-methylated to methionine with the aid of tetrahydrofolate (THF) or, alternatively t-Hcy may participate in a transsulfuration sequence to produce cysteine (Barshop, "Homocystinuria," in CECIL'S TEXTBOOK OF MEDICINE 1112 (Bennett *et al.*, editors; W.B. Saunders Co., Philadelphia; 1996). Homocysteine may be detected and its levels may be quantitated by various techniques and assays known in the art, including, for example, the IMx homocysteine assay marketed by Abbott Laboratories, Inc. Moreover, plasma homocysteine has been shown to rise in proportion to the fall in the glomular filtration rate (Arnadottir *et al.*, Scand. J. Clin. Lab. 56:41-46).

Overproduction of homocysteine leads to a condition known as hyperhomocysteinemia. Hyperhomocysteinemia can arise as a result of a genetic defect in the metabolism of homocysteine (t-Hcy) (*e.g.*, congenital homocystinuria) (Monnerat *et al.*, 1997 Schweiz Med. Wochenschr. 127: 1489), due to drug or diet induced folic acid deficiency or other vitamin deficiencies, and as a consequence of hemodialysis treatment for end stage renal disease (Quinn *et al.*, 1997 J. Clin. Oncol. 15: 2800). Elevated homocysteine levels have also been associated with histologically confirmed Alzheimer's disease and vascular dementia. Specifically, subjects in the top homocysteine tertile (more than 14 micromoles/L) were 4.5 times more likely to have Alzheimer's than those in the bottom tertile (less than 11 micromoles/L). Subjects in the lowest tertiles for folate and vitamin B12 were 3.3 times and 4.3 times, respectively, more likely to have Alzheimer's disease than those in the top tertiles. (American Medical Association annual Science Reporters Conference on October 19, 1998 at Durham, North Carolina).

Although hyperhomocysteinemia is not confined to ESRD patients, ESRD patients generally have greater elevations in plasma of total homocysteine (263-448% increase in t-Hcy) than patients with renal failure (166-290% t-Hcy) or in other forms of

hyperhomocysteinemia. Data from patients studied indicate that approximately 40% of an oral dose of folate is eliminated in a single dialysis treatment (Foley *et al.*, 1998 J. Am. Soc. Nephrol. 9: 267; Gris *et al.*, 1994; Cressman *et al.*, 1992; and Paul *et al.*, 1993). Each hemodialysis session removes 50-75 μg of folic acid, or about 80% of the normal dietary intake (Foley *et al.*, 1998; Gris *et al.*, 1994; and Cressman *et al.*, 1992). During folic acid therapy, the quantity removed increases with the rise in plasma folate levels (Foley *et al.*, 1998). Dialyzer clearance of folate results in a fall of plasma folate from 46.4 ± 73.3 nmol/L to 25.9 ±30.7 nmol/L (44% decline) (Paul *et al.*, 1993). As a result, hemodialysis patients have a special requirement for very high levels of folate intake merely to maintain a given folate blood plasma level because of hemodialysis-induced loss of folic acid.

Folic acid (also known as folate) is used to treat hyperhomocysteinemia to reduce homocysteine levels, because folate is a cofactor in the transformation of homocysteine to methionine. Both folic acid (1-5 mg/day) alone and folic acid (1-5 mg/day) in combination with vitamins B_6 and B_{12} (also known as cyanocobalamin) have been shown to reduce homocysteine concentrations in plasma (Welch *et al.*, 1998 N. Engl. J. Med. 338: 1042). Patients with mild hyperhomocysteinemia, which is frequently encountered in patients with premature arteriosclerotic disease, have been treated with vitamin B_6, folic acid (5.0 mg/day) and betaine to re-normalize their homocysteine levels in virtually all cases (Franken *et al.*, 1994 Arterioscler. Thromb. 14: 465). In patients with severe hyperhomocysteinemia, treatment with pyridoxine hydrochloride (240 mg/day) and folic acid (10 mg/day) reduced fasting homocysteine levels after 4 weeks by a mean of 53% (Brattstrom *et al.*, 1990 Atherosclerosis 81: 51). However, some researchers have indicated that merely providing vitamins (folic acid, vitamin B_{12} and vitamin B_6), will not rectify severe hyperhomocysteinemia, and that other homocysteine modulating agents, such as

methioninase, need to be administered to control severe hyperhomocysteinemia (Lishko *et al.*, 1998 U.S. Patent No. 5,715,835).

Although folic acid is a powerful homocysteine lowering agent in subjects with normal kidney function, it is much less powerful in those with kidney failure and ESRD. Folic acid and vitamins B_6 and B_{12} are indicated for optimal homocysteine metabolism (Ueland *et al.*, "Plasma homocysteine and cardiovascular disease," IN ATHEROSCLEROTIC CARDIOVASCULAR DISEASE, HEMOSTASIS AND ENDOTHELIAL FUNCTION 183 by R. B. Francis ed., Marcel Dekker, New York 1992; Mayer *et al.*, 1996 J. Am. Coll. Cardiol. 27: 517). Plasma t-Hcy concentrations normally correlate negatively with plasma concentrations of these vitamins (Robinson *et al.*, 1996 Circ. 94: 2743; Robinson *et al.*, 1995 Circ. 92: 2825). Moreover, plasma t-Hcy levels can be elevated to those levels found in renal failure in normal subjects who are severely deficient in these vitamins (Guttormsen *et al.*, 1996 J. Clin. Invest. 98: 2174, Stabler *et al.*, 1998 J. Clin. Invest. 81: 466). However, unlike in folate resistant ESRD patients, t-Hcy levels in those with normal renal function and vitamin deficiency can be easily corrected by modest replacement of folic acid, vitamin B_6 and vitamin B_{12} (Guttormsen *et al.*, 1996). Nephrologists have recognized that patients with renal failure are at risk for folic acid and B vitamin deficiency. Therefore, physicians routinely provide modest vitamin B supplementation to ESRD patients and patients with renal failure. One widely used B vitamin supplement, Nephrocaps, contains 1 mg folic acid, 10 mg pyridoxine, and 6 μg cyanocobalamin. This multi-vitamin is recommended to be taken once daily.

A daily folic acid supplement of 0.65 mg/day is sufficient to normalize mild to moderate hyperhomocysteinemia in most individuals with normal renal function. In patients with severe renal failure, somewhat higher folate dosages have been used (1-5 mg). In one study (Guttormsen *et al.*, 1996), supplements of folic acid were given to patients with normal

renal function and hyperhomocysteinemia due to folate deficiency. In these patients, 0.2 mg of folic acid increased the plasma folate concentrations 2.1-fold, whereas 5 mg daily elevated plasma folate levels above the limit of measurement. Indeed, in another study (Guttormsen et al., 1996 Am J. Clin. Nutri. 63: 194), in normal subjects with deficiencies of folic acid and vitamin B_{12}, 5 mg daily of folic acid achieved the same decrease in t-Hcy as was achieved with one fiftieth of this dose in the earlier study. Presumably, in subjects with normal renal function, 0.1 to 0.2 mg of folic acid daily is near maximal for lowering t-Hcy levels.

Most interventions wherein folic acid is administered to treat hyperhomocysteinemia use 1.0 mg/day or less of folic acid (Allen, 1998 U.S. Patent No. 5,795,873; Trimbo *et al.*, 1998 U.S. Patent No. 5,728,678). One liquid nutritional supplement recommended for patients with ESRD contains, in addition to other vitamins and minerals, 1.0 mg/day folic acid (Mulchandani *et al.*, 1992 U.S. Patent No. 5,108,767). However, tropical sprue, a condition that is unrelated to ESRD, has been treated by administering folic acid in an amount of 100 mg/day for 2 weeks or in a single dose of 500 mg (Butterworth *et al.*, 1989 Am. J. Clin Nutr. 50: 353).

In at least one study, supraphysiologic doses of B-vitamins have been used to correct hyperhomocysteinemia in dialysis patients (Bostom *et al.*, 1996 Kidney Int. 49: 147). However in the Bostom *et al.* (1996) study, only one third of the patients treated with 15 mg folic acid were able to lower their plasma t-Hcy by 25-30% and in only 25% of the patients were homocysteine levels normalized. A later study by van Guldener *et al.* (1998) emphasized that the results of Bostom *et al.* (1996) was likely due to the combination of folic acid, vitamin B_6 and vitamin B_{12}, rather than by 15 mg/day of folic acid alone. Guttormsen *et al.* (1996 J. Clin. Invest. 98: 2174) treated 14 hemodialysis patients with 15 mg daily of folic acid for 2 months. These patients were not taking folate before the study and had very high levels of t-Hcy. Again, folic acid therapy normalized t-Hcy in only 38%.

In the study by van Guldener *et al.* (1998), the researchers were unable to reproduce the results of Bostom *et al.*, *e.g.*, the decrease in blood plasma levels of homocysteine in patients treated with 15 mg/day of folic acid alone.

Folic acid supplementation has also been used to treat drug-induced hyperhomocysteinemia. In rheumatoid arthritis patients treated with methotrexate, both 5.0 mg or 27.5 mg of folic acid were shown to inhibit hyperhomocysteinemia induced by methotrexate (Morgan *et al.*, 1998 J. Rheumatol. 25: 441). However, differences in plasma homocysteine levels as between the 5 mg and 27.5 mg dosages of folic acid were negligible (Morgan *et al.*, 1998). Moreover, methotrexate induced hyperhomocysteinemia impacts only one step in homocysteine metabolism, whereas ESRD induced hyperhomocysteinemia impacts homocysteine metabolism at many points, many of which remain to be fully elucidated.

A high t-Hcy plasma concentration resulting from hyperhomocysteinemia is now considered a risk factor for atherosclerosis, occlusive vascular disease and coronary artery disease (Fallest-Strobl *et al.*, 1997 Amer. Family Phys. 6: 1607; Stein *et al.*, 1998 Arch. Intern. Med. 158: 1301; Welch *et al.*, 1998 N. Engl. J. Med. 338: 1042). Folic acid ($\leq$ 1.0 mg) has been administered to patients both to prevent onset or inhibit progression of coronary artery disease resulting from hyperhomocysteinemia (Woodside *et al.*, 1998 Am. J. Clin. Nutri. 67: 858; Brattstrom, 1996 J. Nutr. 126: 1276S). Combined folic acid and vitamin B_6 (also known as pyridoxine hydrochloride) therapy is recommended by some physicians for administration with anticoagulant therapy for patients with arterial and venous occlusive disease (Guba *et al.*, 1998 Am. J. Med. Sci. 315: 279). Vitamin B_6 and folic acid (5.0 mg/day) have effectively treated young patients with arterial occlusive disease (van den Berg *et al.*, 1994 J. Vasc. Surg. 20: 933). The effects of long-term homocysteine-lowering treatment on endothelial function has been of interest to researchers because of its potential

role in athero- and thrombogenesis. Accordingly, studies have been conducted in which patients have been treated with 1, 5, and 15 mg of folic acid. Some investigators recommend 10-15 mg of folic acid for patients receiving chronic hemodialysis, although in practice patients rarely receive more than 1 mg (Helbig *et al.*, 1977 Folia Haematol. Int. Mag. Klin. Morphol. Blutforsch. 104: 242). However, total normalization of plasma homocysteine levels is seldom achieved even with the higher rates of folate administration (van Guldener *et al.*, 1998 Nephrol. Dial. Transplant 13: 106).

Neither the mechanism by which elevated plasma homocysteine leads to cardiovascular disease nor the mechanism whereby homocysteine accumulates in the plasma of patients with renal insufficiency is adequately understood. It is also not clear whether homocysteine itself is injurious or whether it is a marker of cardiovascular injury. However, successful treatment of hyperhomocysteinemia may reduce the prevalence of cardiovascular disease in patients with renal dysfunction. Thus, there remains a need in the art, for a method of treating patients with compromised renal function to decrease their plasma homocysteine and/or dimethylarginine levels to normal.

SUMMARY OF THE INVENTION

The invention relates to methods and compositions for treating diseases that are characterized by folate resistance in a significant patient population, such as end stage renal disease (ESRD) and Alzheimer's disease. More specifically, the invention provides for a method for treating hyperhomocysteinemia in a subject with compromised renal function, comprising administering a folate in an amount that is effective to normalize plasma homocysteine levels in the subject.

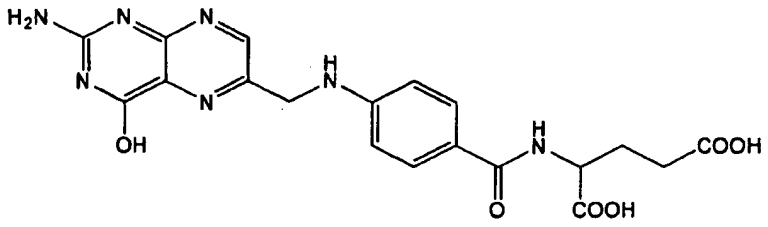
The invention also relates to a pharmaceutical composition for treating hyperhomocysteinemia , comprising at least about 30 mg to about 500 mg of a folate and one

or more of the following: (A) about 1.0 mg to about 150 mg thiamin; (B) about 1.0 mg to about 150 mg riboflavin; (C) about 10 mg to about 500 mg niacin; (D) about 5.0 mg to about 100 mg pantothenic acid; (E) about 100 mcg to about 200 mcg biotin; (F) about 250 mcg to about 2,000 mcg vitamin B_{12}; (G) about 10 mg to about 100 mg vitamin B_6; (H) about 30 I.U. to about 5,000 I.U. vitamin E; (I) about 100 mg to about 2,000 mg vitamin C; (J) about 20 mg to about 200 mg zinc; (K) about 50 mg to about 500 mg iron; and (L) about 300 mg to about 2,000 mg calcium.

The invention further relates to a pharmaceutical composition about 10 mg to about 100 mg of a folate derivative or a folate analog, in combination with at least one of vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, iron, zinc, calcium, pantothenic acid, niacin, riboflavin, thiamine, biotin, a methioninase, a nitrosating agent, Tempol, TEMPO, a thetin and serine.

DETAILED DESCRIPTION OF THE INVENTION

By "folic acid" or "folate" or "pteroylglutamic acid" is meant the chemical with the structure of:



The term "a folate" includes folic acid, its derivatives, analogs, metabolites, optical isomers, and pharmaceutically acceptable salts. By "folate derivative" or "folate metabolite"

is meant the intermediate or active products of a folate, such as would be involved in conversion of homocysteine. These include, for example, 5-methyltetrahydrofolate (also known as 5-methyl-5,6,7,8-tetrahydropteroyl-L-glutamic acid), and 5-formyltetrahydrofolate (also known as folinic acid) and their pharmaceutically acceptable salts, such as magnesium, calcium and disodium salts, and their optical isomers. The terms "folate analog" and "folic acid analog" include compounds with the formula of folic acid and additional substituted groups, such as dihydrofolate, tetrahydrofolate, formiminoglutamate, 5,10-methylenetetrahydrofolate, 10-formyltetrahydrofolate, 5,10-methenyltetrahydrofolate, 5,10-methylenetetrahydrofolate, formiminotetrahydrofolate, hydroxymethyltetrahydrofolate, their pharmaceutically acceptable salts and optical isomers. See Goodman & Gilman's The Pharmacological Basis of Therapeutics 1326-1340 (Ninth Ed. McGraco-Hill, New York, 1996)

By "plasma homocysteine" is meant the total plasma homocysteine ("t-Hcy") measured, whether fasting or not fasting, before or after dialysis. This also refers to an increase in oxidized or reduced forms of protein-bound or free-form homocysteine.

The term "hyperhomocysteinemia" denotes any level of total plasma homocysteine above normal in patients with end stage renal disease ("ESRD") or in patients suffering from other renal disorders that result in elevated homocysteine levels.

The term "folate resistant" or "folate resistance" or "folic acid resistant" or "folic acid resistance" is that population of ESRD patients receiving hemodialysis whose plasma homocysteine level cannot be normalized by administering up to 15 mg of folic acid per day.

The term "vitamin B_{12}" is meant to include "cobalamin." The term "vitamin B_6" is meant to include "pyridoxine."

The term "dialysis" includes hemodialysis as well as other forms of dialysis and similar types of therapy.

The term "compromised renal function" includes renal disorders resulting in elevated plasma homocysteine levels. Renal disorders can include, among others, ESRD and renal failure in a patient not yet receiving dialysis treatment.

The invention provides for a method for treating hyperhomocysteinemia in a subject with compromised renal function, comprising administering a folate in an amount that is effective to normalize plasma homocysteine levels in the subject. Patients who are folate resistant may fall into the category of severe hyperhomocysteinemia or may have minimal to negligible renal function as compared to ESRD patients who respond to ≤ 15 mg of folic acid. Normal total plasma homocysteine concentrations range from 5-15 μmol/L in the fasting state (Welch *et al.*, 1998). Mean values for plasma total homocysteine concentration depend upon plasma creatinine (Dennis *et al.*, 1996 Kidney Int. 50: S11). The preferred patients are those with end stage renal disease and who are receiving dialysis.

The folate to be administered for treating hyperhomocysteinemia may be folic acid, a folate derivative, a folate analog, their pharmaceutically acceptable salts and their optical isomers. Folate derivatives and folate analogs contemplated include 5-methyltetrahydrofolate, 5-formyltetrahydrofolate (also known as folinic acid or Leucovorin), dihydrofolate, tetrahydrofolate, formiminoglutamate, 5,10-methylene-tetrahydrofolate, 10-formyltetrahydrofolate, 5,10-methyltetrahydrofolate, and their pharmaceutically acceptable salts and optical isomers.

The most preferred folate derivative is 5-formyltetrahydrofolate, which is a biologically active metabolite of folate. Leucovorin, a commercially available source of folinic acid, is a mixture of the diastereoisomers of 5-formyltetrahydrofolate. The biologically active compound is the L-isomer. It does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folate as a source of one-carbon moieties. In plasma, it is rapidly converted to 5-methyl-tetrahydrofolate, another

biologically active folate metabolite. While the half-life of L-leucovorin is very short, its biological affect is of longer duration because of the production of active metabolites. Following an oral dose, the peak concentration of reduced folate is seen at 2.3 hours with a terminal half-life of 5.7 hours.

Folic acid is preferably administered in an amount of from about 30 mg/day to about 500 mg/day. More preferred ranges of folic acid for treating hyperhomocysteinemia in patients with ESRD or compromised renal function include: about 40 mg/day to about 400 mg/day; about 50 mg/day to about 200 mg/day; about 30 mg/day to about 100 mg/day; and about 60 mg/day to about 100 mg/day. Folate derivatives and folate analogs are preferably administered in amounts of about 10 mg/day to about 100 mg/day. A more preferred range is from about 10 mg/day to about 50 mg/day.

The invention also pertains to pharmaceutical compositions containing a folate for treating hyperhomocysteinemia. The pharmaceutical compositions described may be formulated to provide immediate or sustained release. Pharmaceutical compositions that may be used to treat hyperhomocysteinemia in a subject with compromised renal function include compositions comprising at least about 30 mg to about 500 mg of a folate and one or more of the following: (A) about 1.0 mg to about 150 mg thiamin; (B) about 1.0 mg to about 150 mg riboflavin; (C) about 10 mg to about 500 mg niacin; (D) about 5.0 mg to about 100 mg pantothenic acid; (E) about 100 mcg to about 200 mcg biotin; (F) about 250 mcg to about 2000 mcg vitamin B_{12}; (G) about 20 mg to about 100 mg vitamin B_6; (H) about 30 I.U. to about 5,000 I.U. vitamin E; (I) about 100 mg to about 2,000 mg vitamin C; (J) about 20 mg to about 200 mg zinc; (K) about 50 mg to about 1,000 mg iron; and (L) about 200 mg to about 2,000 mg calcium (*e.g.*, calcium carbonate).

The invention also provides pharmaceutical compositions that comprise about 10 mg to about 100 mg of a folate derivative or a folate analog, in combination with at least one of

vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, iron, zinc, calcium, pantothenic acid, niacin, riboflavin, thiamine, biotin, a methioninase, a nitrosating agent, Tempol, TEMPO, a thetin and serine.

The invention is related to the discovery that patients with hyperhomocysteinemia induced by ESRD, dialysis or other renal disorders, may be treated with dosages of a folate by itself, or in combination with other vitamins, minerals, amino acids, and/or homocysteine regulating agents. In folate resistant patients, when folic acid is administered in amounts of $\leq$ 15 mg, either alone or in combination with vitamins B_6 and/or B_{12}, the patient's plasma homocysteine level cannot be normalized. The substantially higher dosages of folic acid in any of the proposed compositions described below, are therapeutically effective and are of sufficient concentration to be considered a prescription formulation in the United States.

This invention provides methods and pharmaceutical compositions for reducing elevated plasma homocysteine levels. The pharmaceutical compositions and methods can also be used to reduce the incidence and complications arising from hyperhomocysteinemia, such as vascular and cardiovascular disease (*e.g.*, coronary artery disease, carotid artery disease, peripheral vascular disease, atherosclerosis and occurrences of clotting of the arterio-venous grafts, fistulae, catheters or other devises used to gain blood access for hemodialysis). These compositions comprise a therapeutically effective, dosage of folic acid, its analogs, derivatives, pharmaceutically acceptable salts and optical isomers, which either alone or in combination with other vitamins, minerals, amino acids, and/or antioxidants, can normalize plasma homocysteine levels in folate resistant ESRD patients.

For purposes of this invention, appropriate individuals with "end stage renal disease" or "ESRD" should preferably receive hemodialysis and be folate resistant, as defined above. It is this category of ESRD patient that would be both appropriately and safely treated with the proposed folate therapy. However, folate resistant patients with other renal disorders can

also be administered this therapy to treat elevated plasma homocysteine levels. This folate therapy is preferably in conjunction with other antioxidants, vitamins, minerals, amino acids and/or homocysteine regulating agents.

Pharmaceutical compositions for treating folate resistant ESRD patients include folic acid, its analogs, derivatives, pharmaceutically acceptable salts and optical isomers. The pharmaceutical compositions may contain more than one type of folate, for example, a methyltetrahydrofolate and folic acid. Another preferred pharmaceutical composition used for treating this category of ESRD patient contains one or more folates (*e.g.*, tetrahydrofolate), and other B vitamins, especially either or both vitamin B_6 or vitamin B_{12}.

The vitamin B_6 dosage disclosed in this invention to be used in any pharmaceutical composition containing a folate for treatment of hyperhomocysteinemia and/or hyperdimethylargininemia is in the range of about 2 mg to about 500 mg. The vitamin B_{12} dosage range contemplated is about 1 mcg to about 500 mcg. More preferred ranges to be administered to patients fall in the range of about 5 mg to about 50 mg for B_6 and from about 10 mcg to about 100 mcg for B_{12}.

In addition to folate compositions containing one or more folates and any combination of other vitamins, antioxidants, amino acids, and minerals, the present invention further comprises other homocysteine-modulating agents. Additional homocysteine modulating agents which may preferably be co-administered include nitrosating compounds such as those discussed in Stamler *et al.*, 1995 U.S. Patent No. 5,385,937 (*e.g.*, nitroglycerin, nitric oxide, S-nitrosothiol, S-nitroso-protein, nitroprusside, sydnonimines, furoxans, nitrosonium salts and related compounds); Tempol (4-hydroxy-2,2,6,6-tetramethyl-1-piperidine-1-oxyl) and TEMPO (U.S Application No. 08/933,379 filed September 19, 1997 and PCT application filed September 21, 1998); and methioninase compositions such as those described in Lishko *et al.*, 1998 U.S. Patent No. 5,715,835 (*e.g.*, L-methioninase).

Another class of agents which can be administered in combination with a folate include thetins. A folate and a thetin also can be administered in combination with betaine and choline. Preferred thetins include: dimethylacetothetin and dimethylpropiothetin. Amounts of thetin administered in combination with a folate are likely to be lower than that needed if the thetin was administered alone. Amounts of other homocysteine modulating drugs administered to patients in combination with a folate also would likely be less than the other homocysteine regulating agent alone. In the case of thetins, a thetin and a folate could be additionally combined with choline and betaine, as well as other vitamins and minerals. The thetins used for controlling homocysteine and methods of administration are discussed generally in Garrow, 1997 U.S. Patent No. 5,668,173.

The patient class suffering from a renal disorder, such as ESRD, which may be treated with a folate, include those who are folate resistant. One embodiment of the invention is to treat these patients with a folate only. However, as ESRD is a multi-factor disease and is not caused by the interference of only one enzyme involved with homocysteine metabolism, additional active agents are considered for coadministration to a patient with ESRD or compromised renal function; these patients may or may not be receiving hemodialysis. Other agents include vitamins, as well as other plasma homocysteine regulating agents.

The vitamins which may preferably be co-administered with a folate include thiamin (also known as vitamin B_1), riboflavin, niacin, pantothenic acid, biotin, cyanocobalamin, pyridoxine, vitamin E, vitamin C, other anti-oxidants and other water and fat-soluble vitamins, as may be needed to meet the needs of a specific patient, as determined by the physician. Other homocysteine regulating agents, as discussed above, are also contemplated.

The minerals which may be used in admixture with a folate preferably include iron, calcium and zinc.

The nonessential amino acid serine may also be used in combination with a folate. Serine may be present in an amount sufficient to provide about 1-500 mg/kg/day. There are data implicating an abnormality in the transsulfuratin pathway in the pathogenesis of hyperhomocysteinemia in chronic renal insufficienty. The kidney is a major site for the production of serine from glycine and serine deficiency has been demonstrated in patients with renal failure. (Tizianello *et al.*, 1980 J. Clin. Invest. 65:1162-1172). Serine is required for the metabolism of homocysteine via the transsulfuration pathway. (McCully *et al.*, 1993 Ann. Clin. Lab. Sci. 23:477-493).

The preferred routes for administering compounds of this invention include oral and intravenous. Intravenous administration of the contemplated compounds preferably occurs immediately after a patient has received hemodialysis or other dialysis-like treatment. Tablets, capsules, and liquids can be taken orally, typically during or immediately after hemodialysis. Other methods of administering a folate can also be utilized.

The pharmaceutical compositions discussed above may also be for parenteral, rectal or buccal administration, or in a form suitable for administration by inhalation or insufflation. The pharmaceutical compositions may further be formulated using one or more pharmaceutically acceptable carriers and excipients. The compositions can be administered in a single unit (*e.g.*, one tablet), intravenously, in separate units, in a time release formulation, or orally in separate administrations during the course of a day.

For oral administration, the pharmaceutical compositions may be, for example, tablets or capsules prepared by conventional means, in admixture with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); wetting agents (*e.g.*, sodium lauryl sulphate); glidants;

artificial and natural flavors and sweeteners; artificial or natural colors and dyes; and solubilizers. The pharmaceutical compositions may additionally be formulated to release the active agents in a time-release manner as discussed in U.S. Patent Nos. 4,690,825 and 5,055,300. The tablets may be coated by methods well known in the art.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups, suspensions, or slurries (such as the liquid nutritional supplements described in Mulchandani *et al.*, 1992 U.S. Patent No. 5,108,767), or they may be presented as a dry product for reconstitution with water or other suitable vehicles before use. Liquid preparations of folic acid, and other vitamins and minerals may come in the form of a liquid nutritional supplement specifically designed for ESRD patients. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); preservatives (*e.g.*, methyl or propyl p-hydroxybenzoates or sorbic acid); and artificial or natural colors and/or sweeteners.

For buccal administration, the composition may be in the form of tablets or lozenges.

The active compounds may be formulated for parenteral administration by injection, which includes conventional catheterization techniques or infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may be in the form of suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form for reconstitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The active compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, the active compounds are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient, or as an aerosol spray presentation from a pressurized container or nebulizer, with the use of a suitable propellant (*e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of an active compound and a suitable powder base such as lactose or starch.

For intravenous administration, folate, its analogs, derivatives, as well as other vitamins, minerals homocysteine-modulating agents and antioxidants may be administered as an IV admixture in a suitable isotonic vehicle. Typically intravenous administration of pharmaceutical compositions containing a folate occurs after hemodialysis.

Without further description, it is believed that one of ordinary skill in the art, using the preceding description and the following illustrative examples, can make and utilize the compounds of the present invention and practice the claimed methods. Additionally, all of the preceding pharmaceutical compositions comprising a folate can be used in conjunction with antioxidants, minerals, other vitamins, amino acids, and other agents that modulate plasma homocysteine levels.

The following working examples which disclose compositions containing a folate in combination with minerals, antioxidants, amino acids, and other vitamins, therefore, specifically point out preferred embodiments of the present invention. These examples are not to be construed as limiting the scope of the invention. Other examples involving folic acid, its derivatives and its analogs suitable for reducing plasma homocysteine levels will be apparent to one skilled in the art.

EXAMPLES

In Examples 1-7, folic acid dosages can be varied from about 30 to about 500 mg per tablet, more preferred dosages include from about 30 to about 100 mg in each tablet, capsule or sterile injectable solution. Alternatively, the dosages of folate analogs or folate derivatives, can be varied from 10-100 mg in each tablet, capsule or sterile solution for injection.

Example 1

Cardio-Renal Vitamin Formulation 1 (C•R•V™30)

Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20.0 mg
Pantothenic acid	5.0 mg
Biotin	150 mcg
Cyanocobalamin	500 mcg
Pyridoxine	50.0 mg
Folic acid	30.0 mg

Example 2

Cardio-Renal Vitamin Formulation 2 (C•R•V™60)

Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20.0 mg
Pantothenic acid	5.0 mg
Biotin	150 mcg
Cyanocobalamin	500 mcg
Pyridoxine	50.0 mg
Folic acid	60.0 mg
Zinc	22.5 mg

Example 3

Cardio-Renal Vitamin Formulation 3 (C•R•V™30)

Thiamine	15 mg
Riboflavin	15 mg
Niacin	100 mg
Pantothenic acid	20 mg
Vitamin E	30 I.U.
Cyanocobalamin	500 mcg
Pyridoxine	50 mg
Folic acid	30 mg
Vitamin C	250 mg
Zinc	22.5 mg

Example 4

Cardio-Renal Vitamin Formulation 4 (C•R•V™30)

Thiamine	15 mg
Riboflavin	15 mg
Niacin	100 mg
Pantothenic acid	20 mg
Vitamin E	30 I.U.
Cyanocobalamin	500 mcg
Pyridoxine	50 mg
Folic acid	30 mg
Vitamin C	250 mg
Zinc	22.5 mg

Example 5

Cardio-Renal Vitamin Formulation 5 (C•R•V™30)

Thiamine	15 mg
Riboflavin	15 mg
Niacin	100 mg
Pantothenic acid	20 mg
Vitamin E	30 I.U.
Cyanocobalamin	500 mcg
Pyridoxine	50 mg
Folic acid	30 mg
Vitamin C	250 mg
Iron	105 mg

Example 6

Cardio-Renal Vitamin Formulation 6 (C•R•V™100)

Thiamine	15 mg
Riboflavin	15 mg
Niacin	100 mg
Pantothenic acid	20 mg
Vitamin E	30 I.U.
Cyanocobalamin	500 mcg
Pyridoxine	50 mg
Folic acid	100 mg
Vitamin C	250 mg
Iron	105 mg

Example 7

Cardio-Renal Vitamin Formulation 7 (C•R•V™10)

Thiamine	15 mg
Riboflavin	15 mg
Niacin	100 mg
Pantothenic acid	20 mg
Vitamin E	100 I.U.
Cyanocobalamin	1 mg
Leucovorin calcium	10 mg
Pyridoxine	100 mg
Vitamin C	250 mg

Example 8

Treating a Patient with Folate Resistance with Folate Administered Intravenously

If a patient is not receptive to treatment with folate compositions containing ≤ 15 mg folate either by itself or in combination with other vitamins, minerals and/or antioxidants, then the patient is to be treated as follows. Shortly after concluding hemodialysis, the patient is administered a 60 mg folic acid or 10-100 mg of Leucovorin intravenously in a saline solution, together with, for example, 50 mg vitamin B_6, 25 mg zinc, 500 mcg vitamin B_{12}, 5 mg pantothenic acid, 20 mg niacin, 2.0 mg riboflavin and 1.5 mg thiamin. The patient receives this intravenous administration of folic acid after each hemodialysis session. On days when the patient is not receiving hemodialysis, the patient may be administered a tablet containing the same vitamins and minerals as described above.

In instances wherein the patient's plasma homocysteine level is greater than 100 μmol/L, the intravenous administration of vitamins and minerals are the same except for folic acid which is increased to 100 mg. On days with no hemodialysis, the folate composition may be administered orally.

Example 9

If a patient with a renal disorder not receiving dialysis, has elevated homocysteine levels which cannot be normalized with 15 mg folic acid, then this patient may be treated with any of the compositions discussed in Examples 1-7. If the patient is receiving hemodialysis or a dialysis-like therapy (which compensates for kidney dysfunction by cleansing the blood of accumulating toxins and impurities), then the regimen in Example 8 may be administered to the patient.

All references, articles, texts and patents referred to above are hereby incorporated by reference in their entirety.

CLAIMS

I claim:

1. A method of treating hyperhomocysteinemia in a subject with compromised renal function, comprising administering a folate in an amount that is effective to normalize plasma homocysteine levels in the subject.

2. The method of claim 1, wherein the subject has end stage renal disease and the subject is receiving dialysis.

3. The method of claim 1, wherein the subject is folate resistant.

4. The method of claim 3, wherein the folate is folic acid and is administered to the subject in an amount of about 30 mg/day to about 500 mg/day.

5. The method of claim 1, wherein the folate is a folate analog or a folate derivative selected from the group consisting of dihydrofolate, tetrahydrofolate, formiminoglutamate, 5,10-methylene-tetrahydrofolate, 10-formyltetrahydrofolate, 5,10-methyltetrahydrofolate, formino tetrahydrofolate, hydroxymethyltetrahydrofolate, 5-formyltetrahydrofolate, 5-methyl tetrahydrofolate, their pharmaceutically acceptable salts and their pharmaceutically acceptable optical isomers.

6. The method of claim 5, wherein the folate is a pharmaceutically acceptable salt of 5-formyltetrahydrofolate.

7. The method of claim 6, wherein the pharmaceutically acceptable salt of 5-formyltetrahydrofolate is Leucovorin calcium.

8. The method of claim 5, wherein the folate analog, folate derivative or pharmaceutically active salt or optical isomer thereof, is administered in an amount of between about 10 mg to about 100 mg per day.

9. The method of claim 8, wherein the folate is administered daily.

10. The method of claim 1, wherein the folate is administered orally or intravenously.

11. A pharmaceutical composition for treating hyperhomocysteinemia comprising at least about 30 mg to about 500 mg of a folate and one or more of the following:

(A) about 1.0 mg to about 150 mg thiamin;

(B) about 1.0 mg to about 150 mg riboflavin;

(C) about 10 mg to about 500 mg niacin;

(D) about 5.0 mg to about 100 mg pantothenic acid;

(E) about 100 mcg to about 200 mcg biotin;

(F) about 250 mcg to about 2,000 mcg vitamin B_{12};

(G) about 10 mg to about 100 mg vitamin B_6;

(H) about 30 I.U. to about 5,000 I.U. vitamin E;

(I) about 100 mg to about 2,000 mg vitamin C;

(J) about 20 mg to about 200 mg zinc;

(K) about 50 mg to about 500 mg iron; and

(L) about 300 mg to about 2,000 mg calcium.

12. A pharmaceutical composition for treating hyperhomocysteinemia comprising about 10 mg to about 100 mg of a folate derivative or a folate analog, in combination with at least one of vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, iron, zinc, calcium, pantothenic acid, niacin, riboflavin, thiamine, biotin, a methioninase, a nitrosating agent, Tempol, TEMPO, a thetin and serine.

13. The pharmaceutical composition of claim 12, wherein the composition is formulated to provide a sustained release composition.

14. The pharmaceutical composition of claim 12, wherein the thetin is selected from the group consisting of dimethylpropiothetin and dimethylacetothetin.

15. The pharmaceutical composition of claim 12, wherein the nitrosating agent is selected from the group consisting of nitroglycerin, nitric oxide, S-nitrosothiol, S-nitroso-protein, nitroprusside, sydnonimines, furoxans and nitrosonium salts.

16. The pharmaceutical composition of claim 12, wherein the folate analog or folate derivative is selected from the group consisting of dihydrofolate, tetrahydrofolate, formiminoglutamate, 5,10-methylene-tetrahydrofolate, 10-formyltetrahydrofolate, 5,10-methyltetrahydrofolate, formino tetrahydrofolate, hydroxymethyltetrahydrofolate, 5-formyl tetrahydrofolate, 5-methyl tetrahydrofolate, their pharmaceutically acceptable salts and their pharmaceutically acceptable optical isomers.

17. The pharmaceutical composition of claim 16, wherein the folate is a pharmaceutically acceptable salt of 5-formyl tetrahydrofolate.

18. The pharmaceutical composition of claim 17, wherein the pharmaceutically acceptable salt of 5-formyltetrahydrofolate is Leucovorin calcium.

19. The pharmaceutical composition of claim 18, wherein the folate analog, folate derivative or pharmaceutically active salt or optical isomer thereof, is present in an amount of between about 10 mg to about 50 mg.

20. The pharmaceutical composition of claim 19, further comprising serine.

INTERNATIONAL SEARCH REPORT

Inte...tional application No.
PCT/US00/29788

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/495
US CL : 514/249

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/249

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, CAPLUS, REG, MEDLINE, EMBASE, BIOSIS, PNTTEXT
search terms:folic acid, folate, homocysteine, renal failure, hyperhomocysteinemia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	EP 0 595 005 A1 (VESTA MEDICINES)04 May 1994, see entire text, especially column 4, lines 43-47 and claims 1-6.	1-3, 8-10, 12-13 --- 5-7, 14-20
X	Database CAPLUS, on STN, Accession Number 1995:967908, CHAUVEAU et al. 'Long-term folic acid (but not pyridoxine) supplementation lowers elevated plasma homocysteine level in chronic renal failure'. Miner. Electrolyte Metab. 1995, Vol. 22, No. 1-3, Pages 106-109, see entire abstract.	1-3, 8-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 DECEMBER 2000	Date of mailing of the international search report 01 MAR 2001
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/29788

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARNADOTTIR et al. Treatment with high-dose folic acid effectively lowers plasma homocysteine concentration in cyclosporine-treated renal transplant recipients. Transplantation. 15 October 1997, Vol. 64, No. 7, Pages 1087, see entire text.	1-2, 9
Y	GIRGIS et al. 5-Formyltetrahydrofolate regulates homocysteine remethylation in human neuroblastoma. The Journal of Biological Chemistry. 21 February 1997, Vol. 272, No. 8, pages 4729-4734, see abstract.	1-10
X,P	US 6,054,128 A (WAKAT) 25 April 2000, see claims.	1-3, 8-10, 12-13
Y,P		11,14-20
Y	WO 98/19690 A1 (BRISTOL-MYERS SQUIBB COMPANY) 14 May 1998, see entire text, especially claims.	1-3, 5-20